



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Chemical Safety and
Pollution Prevention

MEMORANDUM

June 30, 2011

SUBJECT: Section 3 registration for new end product to control mosquitoes with the active ingredient S-Metheprene (4.25% ai)

EPA Reg No.: 73049-UTL
CAS No.: 65733-16-5
PC Code: 105401
Decision#: 446771
DP#: 388344
MRID#: 48424301 - 03

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Action Requested

The applicant, Valent Biosciences Corp., has submitted a request for Section 3 Registration for the end use product Metalarv S-PT (Mosquito Growth Regulator Spherical Pellet) containing the insect growth regulation S-Methoprene [REDACTED]. This submission is a continuation of the Experimental Use Permit (73049-EUP-8) for S-Methoprene Pellet 60215.

RECOMMENDATIONS AND CONCLUSIONS

1.0 Executive Summary

The Agency has summarized available acute freshwater fish studies and concluded that S-Methoprene is highly to moderately toxic (0.37-1.52 mg/L) to warm water species and highly to practically non-toxic (0.76-106 mg/L) to coldwater fish. Acute toxicity studies on *Daphnia magna* showed 48 hr EC₅₀ = 0.33 – 0.36 mg/L suggesting that S-Methoprene is highly toxic to freshwater invertebrates on an acute basis. Chronic toxicity to daphnid early life stages showed

effects on growth at 0.051 mg/L. Estuarine/marine invertebrates are more sensitive to S-Methoprene exposure with acute toxicity studies on mysid shrimp at 96 hr LC_{50} = 0.11 mg/L (0.09-0.12 mg/L) and a NOEC = 0.035 mg/L. Chronic studies show that mysid females were very sensitive to S-Methoprene exposure that resulted in significant reduction of young produced after exposure to ≥ 0.002 mg/L. S-Methoprene is non-toxic to plants and is practically non toxic to avian and mammalian species on an acute and chronic basis.

The applicant has submitted an Aquatic Dissipation study, several Product Performance studies, and request for waivers from Non-Target Plant Toxicity Testing. The Aquatic Dissipation study (S-Methoprene Pellets 10 lb/A) showed that residues were **<0.06 – 0.06 ppb on the day of application; 0.078 – 0.294 ppb on one day after application; and <0.06 – 0.088 ppb three days after application.** The residue samples collected between 5 and 35 days post application were below the LOD of 0.06 ppb. Several Product Performance Testing were conducted with the following results:

- 1) Among the four VBC candidate formulations, VBC-60215 performed for 6 weeks post treatment with 7% emergence. Beyond week 6, VBC-60200 outperformed the other formulations;
- 2) VBC-60215 provided high levels of emergence inhibition during two assessment floods when applied up to 41 days pre-flood at 2.5 and 5 lbs/A. The % emergence inhibition was not significantly different between the two pre-treatment timings. The higher rate of 5 lbs/A appears to have provided improved EI during the third assessment;
- 3) Evaluation emergence inhibition of VBC-60215 against *Aedes vexans* at two rates in pre-flood and re-flood study showed no difference in % EI observed between the two rates during the first flood event but there was a statistically significant higher %EI for the 5 lbs/A treatment during the second flooding. VBC-60215 remained efficacious through 28 days of soil exposure plus 28 days of flooding;
- 4) Complete direct application residual studies of VBC-60215 against *Aedes taeniorhynchus* exposure resulted in significantly higher emergence inhibition than the commercial standard on days 28-35 post treatment. This study supports operational **re-treatment intervals for VBC-60215 of 4-5 weeks** at 5 and 10 lbs/A. VBC-60215 provided extended residual emergence inhibition of *Aedes taeniorhynchus* when applied post treatment in microcosms at 5 and 10 lbs/A;
- 5) Evaluation of emergence inhibition of VBC-60215 against *Aedes vexans* at two rates in a flood and re-flood study in the Metropolitan Mosquito Control District showed that VBC-60215 provided very high levels of emergence inhibition of *Aedes vexans* and associated species for 31 days following the 4 lbs/A treatment and support > 30 day re-treatment intervals for this treatment. The study, however, did not provide sufficient to assess the effects of the 2.5 lbs/A rate;
- 6) Evaluation of emergence suppression capacity of VBC-60215 against *Culex quinquefasciatus* at three different water depths in post-flood application in a polluted water microcosm system showed that emergence inhibition of VBC-60215 was significantly higher at water depths of 24 inches or less. This study supports operational re-treatment intervals of VBC-60215 of 4-5 weeks at 10 lbs /A in water up to 24 inches.

The Aquatic Dissipation Study and the Performance Studies are Acceptable. The applicant, also, requested a waiver for Non-Target Plant toxicity testing. After a review of available

information the Agency agrees that a **waiver is Acceptable** for Non-Target Plant toxicity testing. The submitted data is acceptable to support the registration of this product.

1.1 Background

Methoprene is an analog of the juvenile insect hormone (JH) and acts as an insect growth regulator (IGR) with a mode of action that disrupts the growth of target insects at the third instar of development in the pupal stage by acting as a JH III mimic. In a normal insect life cycle, JH III concentration severely decreases at the third instar, allowing the pupa to move to the next instar. The presence of Methoprene interferes with this process and prevents the pupa from proceeding to the adult stage. Since this regulatory mode of action does not result in direct toxicity to target organisms, the Agency considers Methoprene to be a biological pesticide where control of target pests is through disruption of primary gene regulation at the onset of metamorphosis, thus preventing larvae from developing into adults (Hershey *et al.*, 1995, Degitz *et al.*, 2003).

Methoprene is considered to be an effective tool for the control of mosquito populations and the reduction of several mosquito-borne diseases that can result in human health risk. The chemical structure of Methoprene is substantially similar to natural juvenile insect hormone (JH). Both compounds have similar molecular weights and contain long-chain esters with carbon, hydrogen, and oxygen. The major structural difference between the two chemicals is that the juvenile insect hormone has an epoxide ring (cyclic ether) while in Methoprene this ring is converted to an open-chain methyl ether (EPA, 1991).

1.2 Application Rates and Sites of Application for S-Methoprene Pellet 60215

S-Methoprene Pellet 60215 are to be applied evenly over an entire habitat that is flooded and/or is expected to be flooded to maintain continuous control as the site alternate between flood and dry conditions. Sites are listed in table 1.0. Irrigated pastures may be treated after each flooding without the removal of grazing livestock. S-Methoprene Pellet 60215 is to be broadcast applied as a dry product. Application can be made using fixed wing aircraft, helicopters, boat, tractor mounted spreader, handheld or backpack spreader. Apply S-Methoprene Pellet 60215 at any time during the mosquito season. One application will control adult emergence for up to 6 weeks.

Table 1.0 Application Rates and Sites for S-Methoprene Pellet 60215	
Habitat	Rate Range (lbs/acre)
Floodwater Sites	
Pastures, meadows, freshwater swamps and marshes, woodland pools, flood-plains, grassy swales, bogs, tires, and artificial water-holding containers.	2.5 - 5
Dredge spoil sites, waste treatment and settling ponds, ditches, natural and manmade hollows or sinkholes (that retain water).	5 - 10
Permanent Water Sites	
Ornamental ponds and fountains, fish ponds, cattail marshes, water hyacinth beds, flooded	2.5 - 5

crypts, transformer vaults, abandoned swimming pools, tree holes, manmade craters and pits, and artificial and natural water-holding containers.	
Storm drains, catch basins, roadside ditches, cesspools, septic tanks, waste settling ponds, vegetation-choked phosphate pits.	5 -10

2.0 Aquatic Dissipation of S-Methoprene Pellets 60215 (MRID# 484243-01)

Objective: Aquatic dissipation study to generate estimated environmental concentrations (EECs). Determine likely levels of S-Methoprene in surface waters after S-Methoprene 60215 Pellet are applied at proposed application rates.

Methods: Four replicate microcosms (8-ft diameter, 2-ft deep galvanized stock tanks) were filled with 6 inches of soil and 6 inches of water. All four replicates received a pellet application at the maximum label rate (10 lbs/A or about 0.01153 lbs of product per microcosm). The concentration of S-Methoprene is 4.25%ai by weight. One untreated microcosm served as a control. Samples were collected the day before application and 1, 3, 5, 7, 14, 28, and 35 days after treatment. Site location was Orange County, Vermont. Site has installed weather station.

Sampling: Ten samples (10 ml each) were collected into glass jars at equally spaced intervals around the tank to create a composite sample of about 1 liter. To evaluate the potential for S-Methoprene losses during storage and shipping, samples of water were fortified with S-Methoprene in the field and subjected to the same storage and handling as the treatment samples.

Analytical Procedures: Microcosm water samples were analyzed for the presence of S-Methoprene by LC-MS/MS (MRID# 484243-01). Concurrent fortifications of control water with S-Methoprene yielded an average recovery of 97%.

Conclusions: The results of the water analysis (Table IV; MRID# 484243-01) show that residues were **<0.06 – 0.06 ppb on the day of application; 0.078 – 0.294 ppb on one day after application; and <0.06 – 0.088 ppb three days after application.** The residue samples collected between 5 and 35 days post application were below the LOD of 0.06 ppb.

Agency Evaluation of Study: Acceptable

2.1 Product Performance with S-Methoprene Pellets VBC-60215 (MRID#484243-02)

Test 1: Evaluation of emergence suppression capacity of VBC s-methoprene formulations and commercial standard (VBC-60133) against *Aedes aegypti* in microcosm system in Malaysia.

Objective: Evaluate initial efficacy and residual profile of four VBC methoprene formulations and one commercial methoprene formulation against *Aedes aegypti*.

Methods: Plastic tubs (42), each measuring 39.5 cm (L) x 50 cm (B) x 13 cm (D) with surface area of 0.2 m² holding 14 L water per tub. All tubs were layered with 3 *Terminalia cattapa* leaves. Water level in each tub was maintained at 14 L by replenishing evaporated water once every 2 weeks if needed. Each tub was covered with fine mesh netting to prevent colonization of wild mosquitoes and larval predators. Test larvae were laboratory bred *Aedes aegypti*.

Treatment: Each tub was treated with 0.28 g/m² with a pre weighed sample (0.056 g) from VBC.

Sampling: On the day of treatment, fifty L3/L4 *Aedes aegypti* were introduced into each tub. Adult mosquito collection was initiated 72 hours post exposure using a battery operated aspirator. On day 8 post exposure, tubs were cleared of any remaining larvae and pupae. Surviving organisms were transferred into separate cups and observed for emergence. Pupal excuviae were collected and counted from each tub as a cross check. Tubs were then reintroduced with 50 fresh L3/L4 larvae. Efficacy of the formulations was evaluated on a weekly basis.

Analysis: Data was subjected to One-Way ANOVA to confirm means separation. If equality of variance assumptions was homogenous, Tukey HSD was applied; when equality of variances assumption was heterogeneous, Dunnett T3 was applied.

Results: 1) No emergence was observed for week 1 post treatment for all VBC formulations. 2) Emergence remained very low (about 1%) for weeks 2 and 3 post treatment. 3) Week 6 showed the least emergence for treatment using VBC-60215 (6.86 ± 3.20). 4) Lower efficacy was noted for all formulations from week 6 to week 10.

Conclusions: Among the four VBC candidate formulations, VBC-60215 performed the best for 6 weeks post treatment with 7% emergence. Beyond week 6, VBC-60200 outperformed the other formulations (Table 2.0).

Table 2.0. Post Treatment Emergence of Different S-Methoprene Granular Formulations

Formulation	Week 6	Week 7	Week 8	Week 9	Week 10
VBC-60200	18.75 ± 11.07	15.71 ± 6.80	27.60 ± 12.32	21.20 ± 7.68	42.40 ± 10.21
VBC-60214	11.43 ± 5.48	22.00 ± 6.41	41.20 ± 12.55	44.00 ± 16.77	64.40 ± 18.78
VBC-60215	6.86 ± 3.20	23.71 ± 18.38	36.80 ± 11.36	34.40 ± 8.59	53.60 ± 13.03

VBC-60196	31.71 \pm 5.88	45.71 \pm 4.97	68.80 \pm 10.44	65.20 \pm 9.37	68.80 \pm 7.84
VBC-60133	85.14 \pm 3.75	80.57 \pm 4.97	92.40 \pm 2.71	79.60 \pm 4.17	90.40 \pm 3.76

Agency Evaluation of Study: Acceptable

Test 2 & 3: Evaluate emergence suppression capacity of VBC-61215 at two rates in two and five week pre-flood applications in a multiple flood microcosm system

Objective: Evaluate emergence suppression capacity of VBC-61215 at two rates in two and five week pre-flood applications in a multiple flood microcosm system.

Methods: Plastic pools, 0.81 square meters in size, equipped with a drainage system and containing a substrate (sand and topsoil). Three simulated floods with water depths of 6 inches. Pools were drained 4-7 days following each assessment.

Treatment: VBC-60215 was applied at 2.5 and 5 lbs/A. Applications were made to six pools 41 days prior to flooding and another six pools 20 days prior to flooding. An additional six pools served as controls. After a flood event, each pool was infected with several hundred field collected 2nd instar *Aedes vexans* larvae. Adult emergence was monitored by isolating 25 pupae in a one quart jar. Completely empty exuviae were considered proof of emergence. This procedure was repeated weekly for six weeks. Length of study was 10 weeks.

Results: Emergence inhibition during the three time periods is shown in Table 2.0. VBC-60215 showed 100% emergence inhibition (EI) at both rates (2.5 and 5.0 lbs/A) and for both treatment timings during the first assessment flood. During the second assessment flood, the 20 day pre-flood timing resulted in 100% EI at both rates, and the 41-day pre-flood timing resulted in 94% and 98% EI. Lower % EI was observed on the third flood, but pre-flood treatment timing did not appear to affect % EI at either rate (Table 2.1).

Table 2.1 Percent emergence inhibition of VBC-60215 with two pre-flood application timings at two rates after artificial flooding

	Treatment	Rate		First Assessment 24 & 45 DPT	First Assessment 24 & 45 DPT	First Assessment 24 & 45 DPT
1	Untreated			3.3	22.7	6.7
2	VBC-60215 20 days pre	2.5	Lbs/A	100.0	100.0	47.3
3	VBC-60215 41 days pre	2.5	Lbs/A	100.0	94.0	44.7
4	VBC-60215 20 days pre	5.0	Lbs/A	100.0	100.0	68.7
5	VBC-60215 41 days pre	5.0	Lbs/A	100.0	98.0	82.7

Statistical Analysis: The test was a randomized complete block experiment with 6 replicates. The percentage of inhibition of emergence was determined as follows: EI% = 1 – (number of

successfully emerged adults/total number of pupae). Data were subjected to ANOVA and Duncan's Multiple Range Test at 0.05 level of probability.

Conclusions: VBC-60215 provided high levels of emergence inhibition during two assessment floods when applied up to 41 days pre-flood at 2.5 and 5 lbs/A. The % emergence inhibition was not significantly different between the two pre-treatment timings. The higher rate of 5 lbs/A appears to have provided improved EI during the third assessment.

Agency Evaluation of Study: Acceptable

Test 4: Evaluation emergence inhibition of VBC-60215 against *Aedes vexans* at two rates in pre-flood and re-flood study

Methods: Tubs 2.11 ft² in size, containing a substrate of sand and topsoil. Three flooding events were simulated in the system with a water depth of 4-5 inches after each of three events. VBC-60215 was applied at 2.5 and 5 lbs/A to each tub 17 days prior to first flood event. After first and second flooding each tub was infested with fifty to one hundred field collected 2nd instar *Aedes vexans* larvae. Tubs were infested with *Culiseta incidens* during the third flood. Adult emergence was monitored by isolating pupae to a 24 oz container.

Results: Following treatment, several rainfall events resulted in minor flooding in the tubs but tubs were drained quickly after each event. During the first assessment flood (32 days after treatment; 17 days exposure on soil; 15 days of flooding), the 2.5 lbs/A rate resulted in 100% emergence inhibition and the 5 lbs/A rate resulted in 99.5% inhibition. During the second assessment flood (51 days after treatment; 23 days exposure on soil; 28 days after flooding) the 2.5 lbs/A rate resulted in 85% emergence inhibition and the 5 lbs/A rate resulted in 97.5 % inhibition. Statistical analysis was Single factor ANOVA that compared %EI from treatments vs. controls.

Conclusions: No difference in % EI was observed between the two rates during the first flood event but there was a statistically significant higher %EI for the 5 lbs/A treatment during the second flooding. VBC-60215 remained efficacious through 28 days of soil exposure plus 28 days of flooding.

Agency Evaluation of Study: Acceptable

Test 5: Complete direct application residual studies of s-methoprene formulations against *Aedes taeniorhynchus* in microcosm system in California

Methods: Tubs measuring 4.17 ft² and 16 inches deep were lined with 2 centimeters of sandy loam soil and filled with 6 inches of water and rabbit pellets (organic enrichment). VBC-60215 was compared to a commercial standard (VBC-60133; Altosid Pellets) at 5 and 10 lbs/A. After 24 hours post water introduction, each tub (6 reps) was infested with fifty 3rd instar *Aedes taeniorhynchus*. Tubs were re-infested with the same number of larvae on a weekly basis starting

immediately following the first pupal isolation. Adult emergence was monitored by isolating 25 pupae in clean cups containing treated water from each microcosm. Isolated pupae were monitored until they either emerged or died and empty exuviae were indication of emergence success. This procedure was repeated on a weekly basis for six weeks.

Results: VBC-60215 showed 98 – 100% emergence inhibition at both rates for three post initial treatment. After four weeks post treatment, VBC-60215 showed emergence inhibition of $80\% \pm 3.3$ and $88\% \pm 2.7$ for 5 and 10 lbs/A, respectively.

Conclusions: VBC-60215 exposure resulted in significantly higher emergence inhibition than the commercial standard on days 28-35 post treatment. This study supports operational re-treatment intervals for VBC-60215 of 4-5 weeks at 5 and 10 lbs/A. VBC-60215 provided extended residual emergence inhibition of *Aedes taeniorhynchus* when applied post treatment in microcosms at 5 and 10 lbs/A.

Agency Evaluation of Study: Acceptable

Test 6: Evaluate emergence inhibition of VBC-60215 against *Aedes vexans* at two rates in flood and re-flood study in the Metropolitan Mosquito Control District

Objective: Evaluate emergence inhibition of VBC-60215 against *Aedes vexans* at two rates in flood and re-flood study in the Metropolitan Mosquito Control District

Methods: Natural floodwater sites were selected from within the Metropolitan Mosquito Control district where small floodwater sites forming distinct individual pools were assigned treatments in this small plot study. Initial treatments were made when sites were partially flooded and larvae (*Aedes vexans*) were detected. VBC-60215 was applied at 2.5 (n=5 sites) and 4 lbs/A (n=11 sites) on various dates. Untreated controls (n=4) were also monitored. When larvae were detected in the test sites, the pupae (45 – 150 per site) were collected, isolated and returned to the MMCD laboratory for bioassay. Emergence inhibition was based on total count of dead adults on the water surface and dead pupae divided by total pupae isolated. The study length was 7 weeks.

Conclusions: VBC-60215 provided very high levels of emergence inhibition of *Aedes vexans* and associated species for 31 days following the 4 lbs/A treatment and support > 30 day re-treatment intervals for this treatment. The study, however, did not provide sufficient to assess the effects of the 2.5 lbs/A rate.

Agency Evaluation of Study: Acceptable

Test 7: Evaluation of emergence suppression capacity of VBC-60215 against *Culex quinquefasciatus* at three different water depths in post-flood application in a polluted water microcosm system

Objective: Complete direct application studies of VBC-60215 in various water depths against

Culex quinquefasciatus in microcosm system in CA.

Methods: Plastic totes measuring 3.76 ft² and 33 inches deep. water depth was maintained at the following depths: 12 inches, 24 inches, and 33 inches. Rabbit pellets were added for organic enrichment. VBC-60215 was added at the rate of 10 lbs/A at three different water depths. Totes were allowed natural infestation with *Culex quinquefasciatus* mosquito larvae (3rd instar). After isolating 25 pupae in clean emergence containers, all totes were monitored on days 7, 14, 21, 28, 35, and 42 post-treatment for adult emergence. Completely empty exuviae were considered emergence success.

Results: Emergence inhibition exceeded 95% for 4 weeks at water depths of 12 and 24 inches, but only for one week at a depth of 33 inches. At 4 weeks post treatment, VBC-60215 showed emergence inhibition of 98.0% \pm 1.1, 100% and 62.0% \pm 4.0 at water depths of 12, 24, and 33 inches, respectively.

Conclusions: Emergence inhibition of VBC-60215 was significantly higher at water depths of 24 inches or less. This study supports operational re-treatment intervals of VBC-60215 of 4-5 weeks at 10 lbs /A in water up to 24 inches.

Agency Evaluation of Study: Acceptable

3.0 Request for Waiver (Non-Target Plant Testing)

The applicant addressed the waiver requests on Non-Target Plant Toxicity Testing (850.4100, 850.4150) by citing the acute toxicity profiles (S-Methoprene and the non-toxic nature of the other ingredients in the formulation for S-Methoprene Pellet 60215. All of the inerts found in the end product have been used in commercial agricultural products; all are allowed in Nonfood Use Pesticide products and have exemptions from tolerance requirements. The waiver request includes the following studies: Non-Target Plant Seedling Emergence and Non-Target Plant Vegetative Vigor. The available data show that methoprene will not result in unreasonable adverse effects to the environment since the compound degrades rapidly via sunlight, is metabolized in soil and does not leach to ground water. The applicant also presents the argument that methoprene and the inerts associated with the formulation have a low toxicity to mammalian, avian, and aquatic species. The applicant, however, has not presented any information that argues for no toxic effects to plants, especially aquatic species.

In order to expedite this review the Agency's reviewer has presented some information that will show that this compound is not likely to be toxic to plants. Methoprene is biodegradable and nonpersistent even in plants treated with very high rates. **Breakdown in vegetation:** Altosid is biodegradable and nonpersistent, even in plants treated at very high rates. It has a half-life of less than 2 days in alfalfa when applied at a rate of 1 pound per acre (Zoecon Corp. 1974). In rice, the half-life is less than 1 day (Menzie, 1980). In wheat, its half-life was estimated to be 3 to 7 weeks, depending on the level of moisture in the plant (USEPA, 1982). Plants grown in treated soil are not expected to contain methoprene residues. **Breakdown in soil and groundwater:** Methoprene is of low persistence in the soil environment; reported field half-lives are up to 10 days (USEPA, 1982). In sandy loam, its half-life was calculated to be about 10 days (USEPA, 1982). When Altosid was applied at an extremely high application rate of 1 pound per acre, its

half-life was less than 10 days (USEPA, 1982). In soil, microbial degradation is rapid and appears to be the major route of its disappearance from soil (USEPA, 1982, 1991). Methoprene also readily undergoes degradation by sunlight (USEPA, 1991). Methoprene is rapidly and tightly sorbed to most soils (USEPA, 1982) and is slightly soluble in water (Kidd and James, 1991). These properties, along with its low environmental persistence make it unlikely to be significantly mobile. In field leaching studies, methoprene residues were observed only in the top few inches of the soil, even after repeated washings with water (USEPA, 1982, 1991).

Breakdown in water: Methoprene degrades rapidly in water (Kidd and James, 1991). Studies have demonstrated half-lives in pond water of about 30 and 40 hours at initial concentrations of 0.001 mg/L and 0.01 mg/L, respectively (Menzie, 1980). At normal temperatures and levels of sunlight, technical Altosid is rapidly degraded, mainly by aquatic microorganisms and sunlight (Zoecon Corp., 1974).

Request of Waiver from Non-Target Plant Testing is Acceptable.

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